

**Transdermal Estrogen in Older Premenopausal Women With Anorexia Nervosa**

**NCT02475265**

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## PARTNERS HUMAN RESEARCH COMMITTEE DETAILED PROTOCOL

### I. BACKGROUND AND SIGNIFICANCE

Anorexia nervosa (AN) is a prevalent psychiatric disorder affecting up to 1% of college-aged women in the US (1) and an increasing number of women over 30 years of age (2). AN is characterized by self-imposed starvation and is associated with significant medical complications which lead to increased morbidity and mortality (3). Among the many medical co-morbidities associated with AN, the most common is significant bone loss, which can persist despite weight recovery (4). Nearly 50% of women with AN have osteopenia with an additional 30% meeting WHO criteria for osteoporosis (5, 6). Importantly, this severe bone loss is associated with an increased fracture risk. Nearly 30% of women with AN report a history of a fracture (6) and a prospective study demonstrated a 7-fold increased risk of fracture in women with AN compared to age-matched controls (7). Because AN is a chronic disease that can persist despite psychiatric and nutritional counseling, the bone loss and increased fracture risk can persist and lead to lifelong morbidity. A population-based retrospective study demonstrated the cumulative incidence of fracture, even many years after the diagnosis of AN, to be 57% (8). Therefore, finding a treatment for bone loss associated with AN is of critical importance.

Loss of bone mass in AN results from hormonal alterations in response to chronic nutritional deprivation. The body responds to caloric deprivation by trying to prevent unnecessary energy expenditure. During periods of decreased caloric availability, reproduction and growth become less important for the survival of an organism and therefore these processes are down-regulated. In AN, down-regulation of the reproductive axis results in hypoestrogenemia. Low estrogen states, such as menopause, result in increased bone resorption (9) and therefore the hypoestrogenemia characteristic of AN is a significant contributor to the loss of bone mass. This is supported by the fact that duration of amenorrhea is inversely associated with bone mineral density (BMD) in AN (10). Despite this association, initial studies investigating the effects of *oral* estrogen in AN did not show benefit (11, 12). This is hypothesized to be due to the fact that oral estrogen suppresses IGF-I production (13, 14), an important bone trophic factor. Patients with AN are relatively IGF-I deficient and low systemic IGF-I levels are an important mechanism of bone loss in AN. In humans, systemic IGF-I is produced primarily by the liver under the regulation of growth hormone as well as nutritional status. Systemic IGF-I levels in AN are approximately 50% those of normal weight women or weight-recovered women with a history of AN (15). In animal models, systemic and tissue levels of IGF-I are important for the acquisition of bone mass (16-18) and in humans, low systemic IGF-I levels are associated with low BMD in AN (19) and treatment with rhIGF-I leads to small increases in BMD in women with AN (12). Therefore further suppression of IGF-I by oral estrogen may contribute to further bone loss in AN.

Importantly, *transdermal*, physiologic estrogen replacement does not suppress systemic IGF-I production. Oral estrogen has significant hepatic effects and these effects are bypassed when the estrogen is administered transdermally. Importantly, oral but not transdermal estrogen suppresses hepatic IGF-I synthesis; in contrast, *transdermal estrogen* replacement has been shown to increase IGF-I levels in post-menopausal women (13, 20). The dose of estrogen is also an important factor in IGF-I suppression, such that higher doses of oral estrogen have greater

suppressive effects on IGF-I production (14) and therefore, the supraphysiologic doses of estrogen found in oral contraceptive pills suppress IGF-I to a greater degree than physiologic doses of oral estrogen.

Understanding the effects of transdermal estrogen on a population of older, premenopausal women with AN will be critical in order to provide potential treatment options for this population. Although physiologic and supraphysiologic doses of oral estrogen do not increase BMD in women with AN, when compared to placebo (11, 12), physiologic, *transdermal estrogen* replacement increases BMD in adolescent girls with AN in as little as 6 months (21). A randomized, placebo-controlled study of 110 adolescent girls with AN treated predominantly with transdermal, physiologic estrogen demonstrated a 1.8% increase in lumbar spine (LS)BMD after 6 months (compared to a -0.5% loss of LSBMD in the placebo group) (21). LSBMD continued to increase with time, such that a 2.6% increase in LSBMD was observed after 18 months of treatment. Yet these data cannot be extrapolated to adults with AN because adolescence is a time of bone accrual and in contrast to adult women with AN who have high levels of bone resorption (22), adolescent girls with AN are in a state of low bone turnover (23). Therefore, whether transdermal estrogen would have the same effect in adult women with AN is unknown.

## II. SPECIFIC AIMS

**Specific Aim 1: We hypothesize that administration of transdermal estrogen will increase bone mineral density (BMD) and improve parameters of bone microarchitecture and strength in osteopenic women with anorexia nervosa (AN) and that response to treatment will be predicted by markers of bone resorption**

We will investigate in women with AN in a 6-month open-label study whether treatment with transdermal estrogen replacement will increase BMD, improve parameters of bone microarchitecture, as assessed by HR-pQCT and improve bone strength, as estimated by microfinite element analysis and whether these increases are associated with bone turnover markers.

**Specific Aim 2: We hypothesize that administration of transdermal estrogen will decrease vertebral MAT in women with AN and this decrease will be associated with increases in BMD**

We will investigate in women with AN in a 6-month open-label study whether treatment with transdermal estrogen replacement will decrease vertebral MAT, as assessed by <sup>1</sup>H-magnetic resonance spectroscopy, and whether this decrease in vertebral MAT is associated with increases in BMD.

**Specific Aim 3: We hypothesize that MAT levels are associated with changes in physiological endogenous estrogen in healthy women, and that MAT levels will be the highest during the follicular phase of the menstrual cycle and lowest during the luteal phase**

We will investigate in normal-weight women whether changes in endogenous estrogen levels during the follicular and luteal phases of menstrual cycles are associated with changes in MAT levels, as assessed by <sup>1</sup>H-magnetic resonance spectroscopy.

### **III. SUBJECT SELECTION**

We will screen a total of 50 individuals. We will screen 25 individuals 25-50 years of age with AN to study 11 patients for an evaluable 10 patients for six months in this open-label pilot study. We will screen 25 normal-weight individuals 21-40 years of age to study 11 patients for an evaluable 10 patients for two months in the control study. Recruitment will occur in one of 6 ways: 1.) Subjects who have participated or screened in previous studies in the Neuroendocrine Unit and have asked to be notified of future studies will be contacted. 2.) Newspaper advertisements and online advertisements will also be utilized as a potential recruitment strategy. 3.) Subjects may be identified using the RPDR and their physicians will be contacted and provided with information about the study. 4.) RSVP for Health, a registry through which interested individuals can register to receive information about clinical trials, will be used to send emails and mailings to potential subjects. 5.) We will post flyers advertising the study in approved areas of the Massachusetts General Hospital main campus, as well as at gyms and exercise studios in the greater Boston area. 6.) A description of the study will be included on the “Research Studies” page of the Neuroendocrine Clinical Research Program website, available at <http://endocrineweb.wix.com/neuroendocrine-unit#!research-studies/cee5>.

If one of Dr. Fazeli’s own patients is identified as a potential subject, we will contact the patient in writing initially, and allow the patient to contact us if they are interested in participating or finding out more information about the research study.

#### **Inclusion criteria for AN women:**

- Female; ages 25-50 years
- DSM-V psychiatric criteria for AN, and amenorrhea
- T-score of  $\leq -1.5$  at spine or hip
- Treatment team or treatment professional in place for clinical treatment/monitoring during the study

#### **Exclusion criteria for AN women:**

- Diseases known to affect bone metabolism, including untreated thyroid dysfunction, vitamin D deficiency, Cushing’s syndrome, diabetes mellitus or renal insufficiency
- Personal history of venous or arterial clot
- History of stroke or myocardial infarction
- History of migraine headaches
- History of hypercoagulable disorder
- Personal history or history of a first-degree relative with breast cancer
- History of hereditary angioedema
- Any medication known to affect bone metabolism, including systemic glucocorticoids within three months of the baseline visit, depot medroxyprogesterone within 6 months of the baseline visit, oral bisphosphonates within one year of the baseline visit or IV bisphosphonates within three years of the baseline visit\

- Bone fracture within the prior 12 months
- Serum potassium < 3.0 meq/L or serum ALT > 3 times the upper limit of normal
- Fasting serum triglyceride level > 150 mg/dL
- Pregnant or breastfeeding
- Active substance abuse
- The Principal Investigator believes that the subject may not be able to safely complete the study

**Inclusion criteria for normal-weight women:**

- Women between the ages of 21-40 years of age
- 90-110% of ideal body weight
- Regular menstrual cycles (21-35 days in length with no more than 5 days of inter-cycle variability) for at least 12 months
- Normal thyroid function

**Exclusion Criteria for normal-weight women:**

- Diseases known to affect bone metabolism, including untreated thyroid dysfunction, vitamin D deficiency, Cushing's syndrome, diabetes mellitus or renal insufficiency
- Personal history of low bone mass
- Bone fracture within the last 12 months
- Pregnancy or nursing within the last 12 months
- Personal history of an eating disorder
- Contraindications to MRI: cardiac pacemaker, metal implants, claustrophobia
- Medication known to affect bone metabolism -- including systemic glucocorticoids -- within three months of the study. Patients receiving depot medroxyprogesterone (Depo-Provera) will be excluded from participating for one year after their last injection
- History of bisphosphonate use or use of any medication for the treatment of low bone mass
- Active substance abuse

#### **IV. SUBJECT ENROLLMENT**

Informed consent will be obtained from all potential study subjects by a licensed physician investigator no later than at the beginning of the screening visit. At this time, the subject will be explicitly counseled that he or she is free to choose whether or not to participate in this study. Eligible subjects with anorexia nervosa who choose to participate will be started on a transdermal estrogen/progesterone patch (Climara Pro, Bayer HealthCare Pharmaceuticals, Inc) at the baseline visit. All patients will also take a multivitamin which provides 400 IU Vitamin D. Patients who do not meet the RDA for calcium, calcium supplementation will be provided in order to raise subjects' calcium intake to 1,200 mg daily.

If individuals who do not speak English express interest in this study, study investigators will provide a translator who is fluent in both English and the subject's native language and either submit a translation of the entire PHRC approved English version of the informed consent form to the IRB for approval. This is in accordance with the PHRC policy on obtaining and documenting informed consent of subjects who do not speak English.

## **V. STUDY PROCEDURES**

### **Subjects with Anorexia Nervosa**

For the Screening Visit, subjects will be seen at the MGH clinical research center for an outpatient visit to determine eligibility, including medical history/physical exam. History taking will include complete eating disorder, weight, menstrual, medication and fracture histories. The following will also be performed: 1. Blood draw for potassium, BUN/creatinine, ALT, thyroid function studies, FSH, 25-OH vitamin D, triglyceride level 2. Nutritional evaluation, including weight in a gown, height, frame size, calculation of % ideal body weight (% IBW) and BMI, 3. Urine pregnancy test, 4. Bone mineral density (BMD) evaluation by dual energy x-ray absorptiometry (DXA), 5. Verification of the AN diagnosis and lack of comorbid concerns (i.e. suicidality or substance use) will occur by the study psychologist, Dr. Kamryn Eddy.

At the Baseline Visit, the following procedures will be performed: 1. Medical history, physical exam, 2. Urine pregnancy test, 3. Nutritional evaluation, including weight in a gown, height, calculation of % IBW and BMI, and calculation of daily intake of calcium and vitamin D, 4. Exercise history, 5. Blood draw for complete blood count (CBC), reticulocyte count, and hormone analyses including P1NP, osteocalcin, Type I collagen C-telopeptide (CTX), 6. BMD evaluation by DXA (if baseline visit is not within six weeks of screening visit), 7. Bone microarchitecture evaluation by HR-pQCT, 8. MAT assessment by <sup>1</sup>H-MRS, 9. Demonstration for administration of first physiologic transdermal estrogen/progesterone patch (Climara Pro, Bayer HealthCare Pharmaceuticals, Inc) 10. Subjects will receive 400 international units of cholecalciferol daily and 1200mg of elemental calcium daily during the study, 11. Hamilton Anxiety Rating Scale (HAM-A).

At Month 1, Month 2, Month 4, and Month 5 the study doctor will check in with subjects by phone. During a phone check-in, the study doctor will speak with the subject about any changes to the subject's health or medical history since the last time they spoke, and address any questions or concerns the subject may have. If the study doctor discovers anything concerning through a phone check-in, she will make sure that necessary action is taken.

All subjects will return to Massachusetts General Hospital for the Month 3 visit. This visit will include the following procedures: 1. Medical history, physical exam, 2. Urine pregnancy test, 3. Nutritional evaluation, including weight in a gown, height, calculation of % IBW and BMI, 4. Blood draw for CBC, reticulocyte count, and hormone analyses including P1NP, osteocalcin, CTX, 5. BMD evaluation of spine by DXA, 6. MAT assessment by <sup>1</sup>H-MRS, 7. HAM-A.

Month 6 (Final Study Visit): Subjects will undergo: 1. Medical history, physical exam, 2. Urine pregnancy test, 3. Nutritional evaluation, including weight in a gown, height, calculation of % IBW and BMI, 4. Blood draw for CBC, reticulocyte count, and hormone analyses including P1NP, osteocalcin, CTX, 6. BMD evaluation by DXA, 7. Bone microarchitecture evaluation by HR-pQCT, 8. MAT assessment by <sup>1</sup>H-MRS, 9. HAM-A.

Baseline, Month 3, and/or Month 6 visits may be divided into two shorter visits, depending on scan availability; divided visits will be no more than 10 days apart.

### **Normal-Weight Subjects**

For the Screening Visit, subjects will be seen at the MGH Clinical Research Center (CRC) for an outpatient visit to determine eligibility. The screening visit will consist of a medical history, physical exam, laboratory studies, and urine pregnancy test. History taking will include complete weight, menstrual, medication, and fracture histories. The following will also be performed: 1) Blood draw for comprehensive metabolic panel, thyroid function studies, 25-OH vitamin D; 2) Nutritional evaluation, including weight in a gown, height, frame size, calculation of % ideal body weight (% IBW) and BMI; 3) Urine pregnancy test; 4) BMD evaluation by dual energy x-ray absorptiometry (DXA).

Subjects who qualify for the study and choose to enroll will then present for two additional visits: Early Follicular Phase Visit (days 3-5 of the menstrual cycle) and Luteal Phase visit (days 12-21 of the menstrual cycle). At each of these visits, the following procedures will take place: 1) medical history, physical exam; 2) urine pregnancy test; 3) Nutritional evaluation including weight in a gown, height, calculation of % IBW and BMI, and calculation of daily intake of calcium and vitamin D; 4) Hormone analyses including P1NP, osteocalcin, Type I collagen C-telopeptide (CTX); 5) MAT assessment of the lumbar spine (L4) by 1H-MRS.

### **Experimental Endpoints:**

**Nutritional Status:** CRC dietitians will assess the nutritional status of all patients. Metabolic weight and height will be determined and percent ideal body weight and BMI calculated. Activity will be assessed using the Paffenbarger assessment.

**Markers of Bone Turnover:** Sensitive blood and urine markers of bone turnover have been developed to assess bone remodeling in a noninvasive, reproducible fashion. We will measure both markers of bone formation and markers of bone resorption including osteocalcin, P1NP and serum CTX.

**Marrow Adiposity:** Marrow adiposity will be measured by magnetic resonance spectroscopy (MRS). Endpoints will include L4 marrow adiposity, and marrow adiposity of the epiphysis, metaphysis and diaphysis of the femur. Imaging examinations will be performed in the Department of Radiology, Yawkey 6<sup>th</sup> floor or Ellison 2<sup>nd</sup> floor, using a 1.5T or 3.0T MR device (GE Medical Systems, Milwaukee, WI and Siemens, Erlangen, Germany). Magnetic field strengths of 1.5T and 3.0T are approved by the FDA for clinical use. Subjects will be required to lie in a magnet for about 1 hr. The 1.5T and 3.0T MR devices are FDA-approved for clinical use.

**Bone Density:** Bone density will be measured by DXA of the whole body, hip, wrist and lumbar spine (Hologic Discovery A, Hologic, Inc. Waltham, MA) to determine cortical and trabecular bone density. The primary bone mineral density (BMD) endpoint will be the BMD measurement of the PA spine. DXA is a standardized, reproducible method to determine bone density and has been used successfully in our previous studies. It involves minimal radiation exposure to the patient.

**Xtreme CT:** Xtreme CT of the wrist and distal tibia will be performed to evaluate bone density and bone quality by examining the microarchitecture of the bones of the forearm and lower leg and by providing us with information about both the trabecular and cortical components.

**Anxiety:** Evaluation of psychic and somatic anxiety will be performed using the Hamilton Anxiety Rating Scale.

## **VI. BIOSTATISTICAL ANALYSIS**

Change in lumbar spine BMD will be used as the primary endpoint in this study. The lumbar spine is the most frequent and severe site of low BMD in AN (6). We will measure BMD with dual energy x-ray absorptiometry (Discovery A; Hologic, Waltham, MA) at baseline, month 3 and at the final study visit.

We will also be monitoring changes in parameters of trabecular and cortical bone microarchitecture: HR-pQCT will be used to measure volumetric density, morphology and microarchitecture at the distal radius and distal tibia by HR-pQCT (Scanco Medical AG®, Bassersdorf, Switzerland). We will measure trabecular and cortical parameters of bone microarchitecture at baseline and at the final, 6-month study visit.

Additionally, we will assess bone strength by finite element (FE) analysis: Bone strength will be estimated using FE analysis of HR-pQCT scan data using published, validated techniques (54).

We will be measuring change in marrow adiposity of L4 vertebra: Marrow adipose tissue of the L4 vertebra will be measured using <sup>1</sup>H-MRS at baseline, month 3 and at the final study visit.

Statistical Methods: The analyses of these pilot study data will be performed in collaboration with Dr. David Schoenfeld, Professor of Biostatistics at the Harvard School of Public Health and Professor of Medicine at Harvard Medical School, and a study collaborator.

The primary analysis will be a paired t-test comparing the post-transdermal estrogen lumbar spine BMD value to the baseline value. We will study 11 patients for an evaluable 10 patients for 6 months in an open-label study. With 10 patients, we will have a power of over 80% at a two-sided  $p=0.05$  level of significance if the true rate of bone gain is 1.2% (this value is 1/3 less than the percent BMD gain found in adolescent girls treated with physiologic estrogen for 6 months). This calculation assumes a SD of 1.2%.

The secondary analyses will be paired t-tests comparing the post-transdermal estrogen parameters of trabecular microarchitecture, bone strength and marrow adiposity with baseline values. With 10 patients per group, we will have 80% power at a two-sided  $p=0.05$  level of significance if the true rate of difference between the treatments is 1.2%, based on an assumption that the SD is 1.2%. There are currently no data looking at the short-term (6 month) effects of estrogen replacement on bone microarchitecture or strength in postmenopausal women or girls or women with AN and therefore this effect size is an estimate of what might be expected. In postmenopausal women, one year of transdermal estrogen replacement resulted in a 20% decrease in adipocyte volume (as measured by bone biopsy) (55), therefore we believe that a change of 1.2% in marrow adiposity is a conservative estimate of the potential change.



## **VII. RISKS AND DISCOMFORTS**

### **Transdermal Estrogen/Progesterone:**

The transdermal patch is applied weekly and administers 0.045 mg/day of estradiol along with 0.015 mg/day of a 2<sup>nd</sup> generation progestin, levonorgestrel. We believe that a combination patch which includes both estradiol and a progestin is the safest way to administer estradiol to a population of women with an intact uterus. Administration of progesterone is necessary to prevent against endometrial hyperplasia and endometrial cancer and a patch which contains both estradiol and a progestin will prevent the inadvertent use of unopposed estrogen and therefore minimize this risk. Transdermal patches may also be associated with skin irritation at the site of administration but are generally very well tolerated.

The risks of exogenous estrogen and progesterone administration include an increased risk of pulmonary embolus, deep venous thrombosis, stroke, myocardial infarction (MI) and retinal vascular thrombosis (24). We will therefore exclude any subject who has a personal history of a venous or arterial clot, any subject with a history of an MI or stroke, any subject with a history of a hypercoagulable disorder and/or a history of migraine headaches (as exogenous estrogen use in women with migraines may increase the risk of stroke). The Climara Pro patch contains a 2<sup>nd</sup> generation progestin, levonorgestrel, which has a lower thrombotic risk than the 3<sup>rd</sup> or 4<sup>th</sup> generation progestins (25) and replacement-dose transdermal estrogen patches are associated with a much lower risk of venous thromboembolism as compared to oral estrogen replacement and therefore we believe that the transdermal route will minimize the risk of venous thromboembolism (26). An increased risk of invasive breast cancer has been reported with the use of exogenous estrogen as well as an increased risk of abnormal mammograms requiring further evaluation. We will therefore exclude any individual with a personal history or a first-degree relative with a history of breast cancer.

### **Blood Sampling:**

Blood sampling is performed in the study and there is always a very minor risk of infection, bruising, or syncope during a blood draw. There is also the discomfort of having one's blood drawn.

### **Radiation:**

A portion of this research study involves exposure to radiation. The total radiation dose the AN subjects will receive from three or four DXA scans and two HR-pQCT scans is 0.12 milliSievert (mSV). This amount of radiation is approximately 4% of the yearly natural background radiation from the earth and the sky (equivalent to 15 days of background radiation). The total radiation dose the normal-weight subject will receive from one DXA scan is 0.03 mSV. This amount of radiation is less than 1% of the yearly natural background radiation from the earth and the sky (equivalent to 4 days of background radiation). There are no known health risks associated with such a dose.

### **Confidentiality:**

The study staff will stringently protect the confidentiality and privacy of the study subjects by keeping all data in locked offices and/or password protected offices. There is a rare risk of loss of confidentiality but study staff will do our best to prevent

## VIII. POTENTIAL BENEFITS

The AN subjects' bone mineral density may increase during the study, although if it does increase, it is not known how long it will remain increased. We hope and anticipate that the information learned from this study will allow us to better understand the pathophysiology of bone loss in AN and to improve the treatment of patients with bone loss associated with under-nutrition.

There will be no benefits to the normal-weight subjects, but we hope that information learned from this study will allow us to better understand the factors affecting marrow adipose tissue, its association with mineral metabolism, and its possible function in the bone marrow microenvironment.

## IX. MONITORING AND QUALITY ASSURANCE

A number of procedures will be instituted to protect against the potential risks involved in this protocol. All patients will have a pregnancy test on admission prior to being exposed to radiation or receiving study medication and at each study visit thereafter. Bone density measurements will be made three times over the course of 6 months. Xtreme CT measurement will be made two times over the course of six months. The combined radiation from such procedures is less than 1% of the background radiation in one year. Confidentiality of the patients will always be of paramount importance to study investigators. All data on patients will be kept in confidential study binders accessed by only study investigators in locked rooms. No data on study subjects will be shared with persons other than those directly involved in the study, except at the documented request of the patient. Counseling will be made available by a study psychiatrist or psychologist.

A Safety Monitor will be established at MGH. The Monitor will consist of study investigators and an independent clinical expert in mineral metabolism at MGH, Dr. Lisa Nachtigall. The study investigators will review the safety data weekly to ensure the safety of the participants in the study and the Monitor will meet every six months, or more often as needed. The Monitor will review all study procedures, all adverse events, study violations, exceptions and deviations as well as study inclusion and exclusion criteria. The Monitor will review all safety monitoring blood tests and study data as available. All study related serious adverse events will be reported to the Monitor within 24h of occurrence. All unanticipated problems, including adverse events will be reported to the Partners Human Research Committee (PHRC) according to Partners reporting policy and to the FDA according to Title 21 of the Code of Federal Regulations. All unanticipated problems, including adverse events will be reported to the Safety Monitor and the report of the Monitor meetings will be submitted to the Partners Human Research Committee semi-annually.

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